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Short communication

Forage management effects on protein and fiber fractions, protein degradability, and dry matter yield of red clover conserved as silage

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ABSTRACT

Due to the action of *o*-quinones formed via polyphenol oxidase, conserved red clover (*Trifolium pratense* L.) contains abundant rumen undegradable protein (RUP), but inadequate rumen degradable protein (RDP) for dairy cattle. This study examined how forage management influences RDP, RUP, crude protein (CP) and fiber fractions in red clover silage and dry matter (DM) yields during the first full production year. In 2003 and 2006, red clover was cut early on 7 June or late on 14 June with two ~40 d regrowth cuts. For comparison, alfalfa (*Medicago sativa* L.) was cut early on 26 May with three ~32 d regrowth cuts or late using the same schedule as early cut red clover. After ensiling at 370 g/kg DM, CP and fiber were fractionated according to the Cornell Net Carbohydrate and Protein System (CNCPS). RDP and RUP were calculated from CNCPS fractions or estimated by a more convenient *Streptomyces griseus* protease procedure, which gave highly related ($R^2 \geq 0.95$) estimates of CP degradability. Early cutting of red clover favorably decreased RUP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) and increased RDP and CP in the first two harvests. Regression analyses indicated desirable levels of RDP (>150 g/kg DM) and NDF (~400 g/kg DM) could be obtained by harvesting red clover at a mean stage weight maturity of 2.0 at first cut, 3.0 at second cut, and 3.5–4.0 at third cut. Averaged across harvest schedules, red clover had lower RDP, CP and ADF, higher RUP, and similar NDF

Abbreviations: ADF, acid detergent fiber; ADIP, acid-detergent insoluble protein; B1, buffer-soluble protein precipitated by tungstic acid; B2, buffer-insoluble protein extracted with neutral detergent; B3, neutral detergent insoluble protein extracted with acid detergent; CNCPS, Cornell Net Carbohydrate and Protein System; CP, crude protein; DM, dry matter; NDF, neutral detergent fiber; NDIP, neutral-detergent insoluble protein; MSW, mean stage weight; RDP, rumen-degradable protein; RUP, rumen-undegradable protein.

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compared to alfalfa. Total DM yields of red clover and alfalfa were not influenced by harvest schedule. Time of establishment had little effect on silage quality, but it did influence total DM yields; April vs. August seeding depressed red clover yields (12.3 t/ha vs. 13.1 t/ha) but enhanced alfalfa yields (12.5 t/ha vs. 11.9 t/ha) during the first full production year. Overall, these results suggest protein utilization and performance of dairy cattle could be moderately improved without sacrificing DM yield by taking a very early first cutting of red clover followed by regrowth cuttings made at progressively later maturities.

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1. Introduction

With adequate soil moisture, dry matter (DM) yields of red clover can equal or exceed alfalfa in many temperate regions (e.g. [Topp and Doyle, 2004](#)). Red clover also boasts superior fiber digestibility and greater rumen undegradable protein (RUP) than alfalfa, but milk yields from cattle fed red clover diets are usually below expectations ([Broderick et al., 2001, 2007](#)). Coupling of amino acids to *o*-quinones formed via polyphenol oxidase – responsible for low proteolysis in red clover ([Sullivan and Hatfield, 2006](#)) – increases fecal nitrogen excretion ([Broderick et al., 2007](#)), suggesting that intestinal amino acid absorption may be depressed. Poor performance with red clover may also occur because excessive RUP leaves insufficient rumen degradable protein (RDP) for the synthesis of rumen microbial proteins, a vital source of dietary protein for cattle ([Brito et al., 2007](#)).

Previous work with three-cut harvest systems indicated bud-stage red clover from spring growth was lower in crude protein (CP) and higher in neutral detergent fiber (NDF) than subsequent summer cuttings taken at comparable maturity ([Wiersma et al., 1998](#)). Therefore, earlier cutting of spring growth may improve CP and NDF concentrations and their uniformity across harvests. Harvest management effects on RDP and RUP in red clover are not known. It is also not known whether spring vs. late summer establishment influences the maturity, quality and DM yield of red clover during the subsequent production year.

The primary objective of this study was to evaluate how timing of first cutting influences RDP, RUP, CP, and fiber constituents in red clover silage and its DM yield during the first full production year. A secondary objective was to assess how time of establishment and harvest schedule during the first full production year influences the aforementioned silage quality parameters and total DM yield of red clover relative to alfalfa.

2. Materials and methods

In 2002 and 2005 near Prairie du Sac, Wisconsin, red clover and alfalfa were no-till seeded in mid April or in early August after harvest of winter wheat (*Triticum aestivum* L.) grain and straw. Factorial combinations of forage species and time of seeding were assigned to plots according to a randomized complete block design with four replications. Nutrient levels in the soil (Fine-silty, mixed, superactive, mesic Typic Argiudolls) and pests were managed according to Wisconsin Cooperative Extension recommendations.

Forages were cut 5 cm above ground level with a sickle bar mower. During establishment, April-seeded forages were cut two or three times; total yearly DM yields averaged 8.4 t/ha for red clover and 6.5 t/ha for alfalfa. August-seeded forages were not harvested during establishment. During first full production years, red clover subplots were cut early on 7 June or late on 14 June and two additional cuts were taken after 40 ± 4.1 d (mean \pm standard deviation) of regrowth. Alfalfa subplots were cut early on 26 May with three regrowth harvests taken after 32 ± 5.9 d or late following the same schedule as early cut red clover. Mean stage weight (MSW) was used to estimate plant maturity at harvest ([Kalu and Fick, 1981](#); [Ohlsson and Wedin, 1989](#)). Roll-conditioned herbage was wilted for ~24 h in forced-

draft ovens (32 °C day, ambient night temperatures), coarsely chopped, and ensiled in 1 L glass jars for 90 d at ~25 °C (Grabber, 2009). Silages were freeze-dried and ground with a cyclone mill using a 2-mm screen.

Water extracts of silages (Owens et al., 1999) were analyzed for pH and for $\text{NH}_4\text{-N}$ and free amino acids by flow-injection analysis (Broderick and Kang, 1980). Ground samples were analyzed for DM, CP ($6.25 \times \text{N}$ by combustion), and for borophosphate buffer-soluble CP and tungstic acid precipitable B1 protein as described previously (Grabber, 2009). Free peptides were estimated by subtracting free amino acids, ammonia, and B1 protein from buffer-soluble CP. Ground samples in filter bags were sequentially extracted with detergents and analyzed by combustion for N to estimate neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent insoluble protein (NDIP), and acid detergent insoluble protein (ADIP) as described in an earlier study (Grabber, 2009). Fraction B2 protein was estimated as borophosphate insoluble CP minus NDIP and fraction B3 protein was estimated as NDIP minus ADIP. Silage RDP and RUP were calculated according to the Cornell Net Carbohydrate and Protein System (CNCPS), using rate constants of 1.5 h^{-1} for B1, 0.11 h^{-1} for B2, and 0.0175 h^{-1} for B3 and a ruminal passage rate of 0.06 h^{-1} (Sniffen et al., 1992) or estimated by incubating samples containing 8 mg of N with 2 units of *Streptomyces griseus* protease for 16 h (Grabber, 2009).

Relationships between protease vs. CNCPS estimates of RDP and RUP were tested by regression analyses using data averaged across field replicates. Regressions were tested for intercepts of zero and slopes of 1.0 using the TEST statement of PROC REG (SAS, 2003). Data were analyzed using PROC MIXED (SAS, 2003). Seeding time, species, harvest schedule, cutting, year, and their interactions were considered fixed effects and blocks within years and associated interactions were considered random effects. Pair-wise comparisons of least square means by the PDIF procedure (SAS, 2003) were performed if *F*-tests were significant ($P \leq 0.05$). Due to the differing timing and number cuts for red clover and alfalfa, total yearly DM yield and yield-weighted compositional means were compared rather than data from individual cuttings. Main effects and interactions described in the text were significant at $P \leq 0.05$.

3. Results and discussion

During the first full production year, red clover was cut three times between spring and late summer under two managements intended to produce silage containing ~200 g/kg CP and ~400 g/kg NDF which would be suitable for lactating cattle (NRC, 2001). To boost CP and possibly RDP in red clover and to improve its nutritional uniformity across cuttings, an “early” harvest management was initiated when spring growth reached a late vegetative growth stage around MSW 2, while a more typical “late” harvest management was initiated one week later. Two additional cuttings of red clover were taken after ~40 d regrowth periods. For comparison, early cut alfalfa was cut four times at an average maturity similar to late-cut red clover, while late-cut alfalfa was cut at the same dates as early cut red clover.

Silage CP and fiber were fractionated according to the CNCPS. Concentrations of RDP and RUP on a g/kg DM basis were calculated from CNCPS fractions or estimated by a more convenient *S. griseus* protease procedure. Across species, cuttings, and years ($n = 52$), the protease procedure gave highly related but slightly biased predictions (slopes $\neq 1$ and intercepts $\neq 0$) of calculated RDP ($Y = 0.943_{(\text{SE} = 0.0145)}X + 25.2_{(\text{SE} = 2.44)}$, $R^2 = 0.99$) and RUP ($Y = 0.819_{(\text{SE} = 0.0251)}X - 5.03_{(\text{SE} = 1.562)}$, $R^2 = 0.95$). Coblenz et al. (1999) obtained similar results with *S. griseus* protease for predicting the *in situ* RDP of oven-dried alfalfa, red clover, and grasses.

In 2003, precipitation and temperatures during active forage growth were near long-term norms in April through July, while August was relatively hot and dry (Table 1). Except for a dry June, precipitation in 2006 greatly exceeded long-term norms, whereas temperatures were relatively high in April but near normal during other months. Due presumably to variations in growth environment and the timing of regrowth cuttings, main effects or interactions involving year were often significant, however, they usually involved changes in the magnitude rather than the direction of a response. Therefore, data averaged across years are presented and discussed in the following sections.

Table 1

Monthly average temperature (°C) and total precipitation (mm) during active forage growth in 2003 and 2006 compared to long-term norms.

Month	Precipitation			Temperature		
	2003	2006	30-year norm	2003	2006	30-year norm
April	54	126	80	6.7	10.2	7.8
May	115	172	78	13.6	14.2	14.8
June	93	34	100	19.4	19.1	19.9
July	105	120	97	22.1	22.8	22.2
August	32	150	109	23.1	20.3	20.8

3.1. Individual harvest yields and quality of red clover as influence by harvest system

The first cut of red clover had roughly twofold greater DM yield than regrowth cuttings, but the early harvest schedule shifted some DM production from the first to the third cut and it decreased MSW in the first two cuts and considerably increased MSW in the third cut (Table 2). The earlier harvest schedule decreased silage NDF and ADF and increased CP in the first two cuttings, while the opposite effect was observed in the third cut. Treatment effects on silage DM and pH were significant but small.

Harvest schedule had cut specific effects on all buffer-soluble CP fractions and on most detergent fractions except B2 and B3 in red clover silage (Table 3). Most notably, the early harvest schedule increased the proportion of readily degraded buffer-soluble CP and reduced the proportion of slowly degraded NDIP in the first cutting of red clover. As a result of shifts in protein fractions and CP content, RDP on a DM basis increased in the first two cuts and decreased in the third cut under the early harvest schedule. Harvest schedule had cut specific, but modest effects on RUP in red clover silage. Overall, shifting from a late to an early harvest schedule improved the seasonal distribution of yield and the nutritional value and uniformity red clover silage across cuttings.

Regression analysis of data from both years suggest reasonable levels of calculated RDP (>150 g/kg DM) and NDF (~400 g/kg DM) for lactating dairy cattle (NRC, 2001) could be obtained by harvesting red clover at MSW of roughly 2.0 at first cut, 3.0 at second cut, and 3.5 to 4.0 at third cut (Fig. 1). Taking first and second cuts at these maturities also kept RUP relatively low (≤ 50 g/kg DM), but third cut RUP remained high (≥ 60 g/kg DM) at all plant maturities. For growers, these target MSW values correspond to taking a first cut at a late vegetative growth stage with buds just appearing on a few plants, taking a second cut when most plants are bud-stage with flowers just appearing, and taking a third cut when roughly one-third of plants are in bloom.

Table 2

Mean stage weight (MSW), dry matter (DM) yield (t/ha), and silage DM (g/kg fresh weight), pH, neutral detergent fiber (NDF, g/kg DM), acid detergent fiber (ADF, g/kg DM), and crude protein (CP, g/kg DM) in early or late-cut red clover.

	Cut 1		Cut 2		Cut 3		SEM	Cut (C)	Time (T)	C X T
	Early	Late	Early	Late	Early	Late				
MSW	2.17 ^c	2.76 ^b	1.94 ^c	3.19 ^a	3.48 ^a	1.43 ^d	0.119	NS	NS	***
Yield	6.46 ^b	7.19 ^a	3.36 ^c	3.47 ^c	2.72 ^d	2.15 ^e	0.102	***	NS	***
DM	361 ^b	372 ^{ab}	368 ^{ab}	380 ^a	363 ^b	376 ^{ab}	7.3	NS	**	NS
pH	4.18 ^c	4.19 ^c	4.38 ^a	4.20 ^c	4.31 ^b	4.27 ^b	0.026	***	**	***
NDF	380 ^b	426 ^a	381 ^b	416 ^a	419 ^a	369 ^b	6.0	NS	NS	***
ADF	280 ^b	311 ^a	269 ^b	301 ^a	281 ^b	230 ^c	6.1	***	NS	***
CP	205 ^d	181 ^f	236 ^b	198 ^e	216 ^c	248 ^a	2.6	***	***	***

a–f means within rows with unlike superscripts differ ($P \leq 0.05$).

NS, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

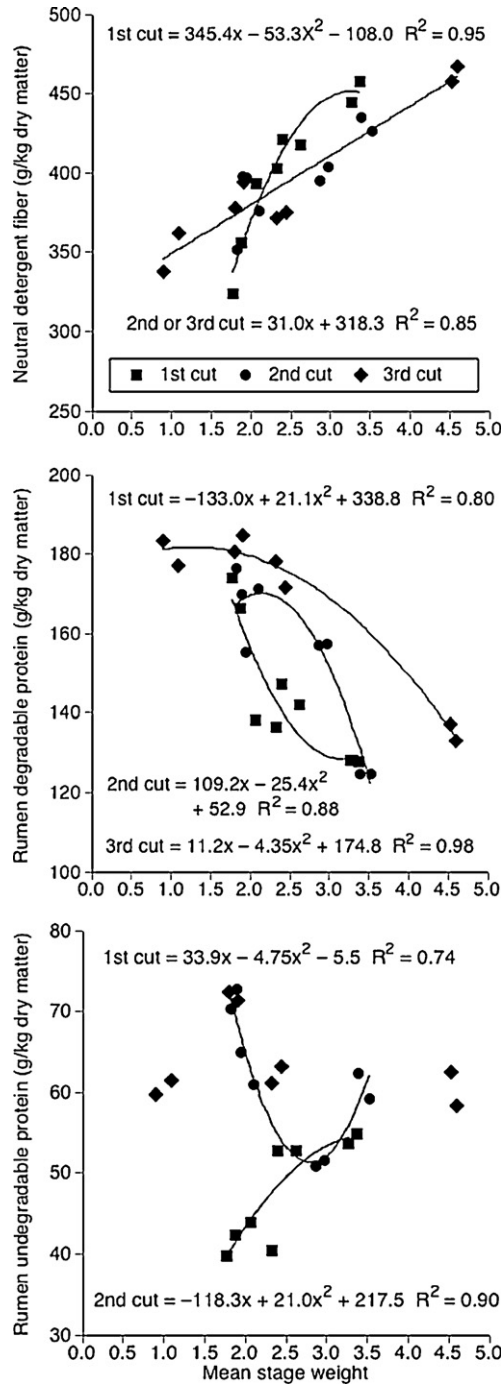


Fig. 1. Neutral detergent fiber, calculated rumen degradable protein, and calculated rumen undegradable protein vs. mean stage weight of red clover. Displayed regressions are significant at $P \leq 0.05$.

Table 3

Protein fractions (g/kg crude protein) and estimates of rumen degradable and undegradable protein (RDP and RUP, g/kg dry matter) in early or late-cut red clover silage.

Constituent [†]	Cut 1		Cut 2		Cut 3		SEM	Cut (C)	Time (T)	C×T
	Early	Late	Early	Late	Early	Late				
NH ₄ -N	34 ^b	39 ^{ab}	43 ^a	30 ^b	39 ^{ab}	24 ^c	2.2	NS	**	***
Free amino acids	281 ^a	265 ^{ab}	277 ^a	250 ^b	233 ^c	240 ^{bc}	6.7	***	NS	*
Free peptides	185 ^a	151 ^b	145 ^b	140 ^b	176 ^{ab}	201 ^a	13.5	*	NS	*
Fraction B1	80 ^{ab}	81 ^{ab}	60 ^b	100 ^a	69 ^{ab}	56 ^b	13.8	NS	NS	*
Total buffer soluble	580 ^a	535 ^b	525 ^{bc}	520 ^c	516 ^c	523 ^{bc}	6.8	***	*	***
Fraction B2	258	263	240	247	251	274	10.5	NS	NS	NS
NDIP	162 ^c	201 ^b	236 ^a	232 ^a	232 ^a	203 ^b	7.8	***	NS	***
Fraction B3	110 ^b	138 ^b	163 ^{ab}	163 ^{ab}	172 ^a	162 ^{ab}	9.6	**	NS	NS
ADIP	52 ^{bc}	63 ^{ab}	72 ^a	70 ^{ab}	60 ^{abc}	43 ^c	6.8	*	NS	*
Calculated RDP	159 ^c	134 ^e	169 ^b	142 ^d	156 ^c	182 ^a	2.5	***	***	***
Protease RDP	139 ^c	111 ^e	153 ^b	125 ^d	136 ^c	162 ^a	2.0	***	***	***
Calculated RUP	46 ^d	48 ^d	67 ^a	55 ^c	61 ^b	66 ^a	1.0	***	NS	***
Protease RUP	66 ^d	70 ^c	83 ^{ab}	73 ^c	80 ^b	85 ^a	1.7	***	NS	***

a–e means within rows with unlike superscripts differ ($P \leq 0.05$).

NS, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

[†] See abbreviations.

3.2. Overall yield and quality of red clover vs. alfalfa

Total DM yields of red clover and alfalfa during the production year were similar and not influenced by harvest schedule (Table 4), but they were influenced by time of seeding; April vs. August seeding depressed red clover yields and maturity (12.3 t/ha vs. 13.1 t/ha, 2.47 MSW vs. 2.59 MSW), but increased alfalfa yields and maturity (12.5 t/ha vs. 11.9 t/ha, 3.35 MSW vs. 3.04 MSW). Effects of seeding time on CP, fiber, or other silage quality parameters were very small or not significant (data not shown).

Although the average MSW of late-cut red clover was similar to early cut alfalfa, early cut red clover had fiber levels that corresponded more closely to early cut alfalfa, while its CP levels more closely matched late-cut alfalfa (Table 4). Silage DM was similar for both species, but pH was lower for red clover than for alfalfa. Harvest schedule effects on MSW and on silage DM, pH, NDF, ADF and CP were significant and often more pronounced in alfalfa than in red clover.

Except for comparable levels of B1 and ADIP, red clover silage had much less buffer-soluble CP components and considerably more detergent-soluble B2 and B3 proteins than alfalfa silage (Table 5). Based on these CNCPS fractions, red clover silage on average had 22% less RDP and 69% more RUP than alfalfa silage. Harvest schedule had a smaller influence on protein fractions and thus early cutting increased RDP levels by only 11% in red clover and 17% in alfalfa with little or no effect on RUP. Even

Table 4

Mean stage weight (MSW), dry matter (DM) yield (t/ha), and silage DM (g/kg fresh weight), pH, neutral detergent fiber (NDF, g/kg DM), acid detergent fiber (ADF, g/kg DM), and crude protein (CP, g/kg DM) in early or late-cut red clover and alfalfa, averaged across harvests.

	Red clover		Alfalfa		SEM	Species (S)	Time (T)	S×T
	Early	Late	Early	Late				
MSW	2.41 ^c	2.65 ^b	2.61 ^b	3.78 ^a	0.052	***	***	***
Yield	12.5	12.8	12.1	12.4	0.25	NS	NS	NS
DM	365 ^c	375 ^{ab}	368 ^{bc}	379 ^a	5.1	NS	*	NS
pH	4.26 ^c	4.20 ^d	4.54 ^b	4.61 ^a	0.023	***	NS	***
NDF	394 ^c	414 ^b	379 ^d	440 ^a	2.7	NS	***	***
ADF	280 ^d	296 ^b	288 ^c	344 ^a	2.7	***	***	***
CP	214 ^b	196 ^c	241 ^a	210 ^b	1.6	***	***	***

a–d means within rows with unlike superscripts differ ($P \leq 0.05$).

NS, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 5

Protein fractions (g/kg crude protein) and estimates of rumen degradable and undegradable proteins (RDP and RUP, g/kg dry matter) in early or late-cut red clover and alfalfa silage, averaged across harvests.

Constituent [†]	Red clover		Alfalfa		SEM	Species (S)	Time (T)	S×T
	Early	Late	Early	Late				
NH ₄ -N	38 ^c	35 ^c	53 ^b	65 ^a	2.5	***	*	***
Free amino acids	266 ^b	258 ^b	370 ^a	357 ^a	7.6	***	NS	NS
Free peptides	177 ^c	155 ^c	240 ^b	288 ^a	10.4	***	NS	***
Fraction B1	73 ^a	80 ^a	88 ^a	30 ^b	7.6	**	*	***
Total buffer soluble	547 ^c	530 ^d	750 ^a	740 ^b	4.7	***	**	NS
Fraction B2	250 ^a	258 ^a	162 ^b	147 ^c	6.6	***	NS	**
NDIP	202 ^b	212 ^a	88 ^d	114 ^c	3.4	***	***	*
Fraction B3	142 ^b	151 ^a	48 ^d	58 ^c	3.1	***	***	NS
ADIP	60 ^a	61 ^a	39 ^b	56 ^a	3.0	***	**	**
Calculated RDP	159 ^c	143 ^d	209 ^a	179 ^b	1.5	***	***	***
Protease RDP	140 ^c	122 ^d	195 ^a	167 ^b	1.4	***	***	***
Calculated RUP	55 ^a	53 ^b	32 ^c	32 ^c	0.5	***	**	*
Protease RUP	74 ^a	74 ^a	46 ^b	43 ^c	0.9	***	*	*

^{a–d} means within rows with unlike superscripts differ ($P \leq 0.05$).

NS, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

[†] See abbreviations.

with early cutting, RDP in red clover was still about 20 g/kg DM less than a much more mature late-cut alfalfa. Thus, cutting management alone cannot shift what might be excessive RUP in red clover to RDP needed for microbial protein synthesis (Brito et al., 2007; Broderick et al., 2007).

In conclusion, these results suggest that taking a late vegetative first cutting of red clover followed by regrowth cuttings made at progressively later growth stages may be an effective strategy for producing silage with reasonable concentrations of calculated RDP (>150 g/kg) and NDF (~400 g/kg). Even with early cutting, however, RDP in red clover was lower than a relatively mature alfalfa, thus rumen microbial protein synthesis in cattle fed red clover based diets may still be impaired unless RDP supplements are fed. Finally, early cutting of red clover improved the seasonal distribution of yield without sacrificing total yearly DM yields, but this must be confirmed with additional long-term studies in other growth environments.

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References

- Brito, A.F., Broderick, G.A., Olmos Colmenero, J.J., Reynal, S.M., 2007. Effects of feeding formate-treated alfalfa silage or red clover silage on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. *J. Dairy Sci.* 90, 1392–1404.
- Broderick, G.A., Brito, A.F., Olmos Colmenero, J.J., 2007. Effects of feeding formate-treated alfalfa silage or red clover silage on the production of lactating dairy cows. *J. Dairy Sci.*, 1378–1391.
- Broderick, G.A., Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63, 64–75.
- Broderick, G.A., Walgenbach, R.P., Maignan, S., 2001. Production of lactating dairy cows fed alfalfa or red clover at equal dry matter or crude protein contents in the diet. *J. Dairy Sci.* 84, 1728–1737.
- Coblentz, W.K., Abdelgadir, I.E.O., Cochran, R.C., Fritz, J.O., Fick, W.H., Olson, K.C., Turner, J.E., 1999. Degradability of forage proteins by in situ and in vitro enzymatic methods. *J. Dairy Sci.* 82, 343–354.
- Grabber, J.H., 2009. Protein fractions in forage legumes containing protein-binding polyphenols: freeze-drying vs. conservation as hay or silage. *Anim. Feed Sci. Technol.* 151, 324–329.
- Kalu, B.A., Fick, G.W., 1981. Quantifying morphological development of alfalfa for studies of herbage quality. *Crop Sci.*, 267–271.
- NRC, 2001. Nutrient Requirements of Dairy Cattle, 7th edition. Update. Natl. Acad. Press, Washington, DC.
- Ohlsson, C., Wedin, W.F., 1989. Phenological staging schemes for predicting red clover quality. *Crop Sci.* 29, 416–420.
- Owens, V.N., Albrecht, K.A., Muck, R.E., Duke, S.H., 1999. Protein degradation and fermentation characteristics of red clover and alfalfa silage harvested with varying levels of total nonstructural carbohydrates. *Crop Sci.* 39, 1873–1880.
- SAS, 2003. SAS PC Windows Version 9.1.3. SAS Institute Inc., Cary, NC, USA.

- Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G., Russell, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. *J. Anim. Sci.* 70, 3562–3577.
- Sullivan, M.L., Hatfield, R.D., 2006. Polyphenol oxidase and *o*-diphenols inhibit postharvest proteolysis in red clover and alfalfa. *Crop Sci.* 46, 662–670.
- Topp, C.F.E., Doyle, C.J., 2004. Modeling the comparative productivity and profitability of grass and legume systems of silage production in northern Europe. *Grass Forage Sci.* 59, 274–292.
- Wiersma, D.W., Smith, R.R., Mlynarek, M.J., Rand, R.E., Sharpee, D.K., Undersander, D.J., 1998. Harvest management effects on red clover forage yield, quality, and persistence. *J. Prod. Agric.* 11, 309–313.